Jatrophane Diterpenes from the Latex of *Euphorbia obtusifolia* with Inhibitory Activity on the Mammalian Mitochondrial Respiratory Chain

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Abstract

Seven diterpenes isolated of the latex of the *Euphorbia obtusifolia* were evaluated as inhibitors of the NADH oxidase activity in submitochondrial particles from bovine heart. Compound **2**, 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 8,9-diacetate 7-isobutyrate 2,3-bis(2-methylbutyrate), was the most potent inhibitor with an inhibitory concentration (IC_{50}) value of 5.1 ± 0.2 μM. In the present study, some structure-activity trends are suggested for the inhibitory activity of these natural products on the mammalian mitochondrial respiratory chain.

Jatrophane macrocyclic diterpenes have been isolated from various species of the Euphorbiaceae family and are potent antileukemic agents *in vivo* [1], [2], [3]. The first reported member of this group of natural products was jatrophone, isolated from the roots of *Jatropha gossypifolia*, and was found to inhibit DNA transcription [4], [5]. Investigations of its biological activities as a tumor inhibitor *in vivo* [1], [2], [3] have demonstrated that these jatrophanes covalently react with thiol groups on proteins including DNA-dependent RNA polymerase [4]. In mitochondria, oxidation of thiols located in the inner membrane causes dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$) [6], an effect that has also been associated with inhibition of the mitochondrial electron transport chain (loss of $\Delta\Psi_m$) by uncoupling of the respiratory chain [7].

Fig. **1** shows the seven diterpenes isolated from the latex of *E. obtusifolia*. All of them are polyacylated derivatives of the same parent jatrophane macrocyclic framework. Structural features common to all compounds reported here are a ketone carboxyl group at C-14, seven hydroxy groups at C-2, C-5, C-7, C-8, C-9 and C-15, a *trans* double bond between C-11 and C-12, and an *exo*-methylene group at C-6. With the present study using jatrophane polyester derivatives from *E. obtusifolia* we wanted to gain insight into the structure-activity relationships (SAR) of such compounds with particular emphasis on their possible NADH oxidase activity in inverted submitochondrial particles (SMP).

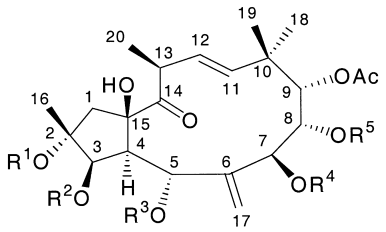

Fig. **1** Jatrophane diterpenes (Ac = acetate; MB = 2-methylbutyrate; iB = isobutyrate; Bz = benzoate; Nic = nicotinate).

Compound	R^1	R^2	R^3	R^4	R^5
1	MB	H	MB	iB	Ac
2	MB	MB	H	iB	Ac
3	MB	MB	H	Ac	Bz
4	iB	H	MB	Ac	Ac
5	Ac	H	MB	iB	Ac
6	MB	H	MB	Ac	Ac
7	Nic	H	MB	Ac	Ac

NADH oxidase activity represents an integrated activity including the respiratory complexes I, II and IV. An NADH oxidase activity assay is thus a good method to establish the SAR of these compounds. As shown in Table **1**, all compounds **1 – 7** inhibited NADH oxidase activity, with IC_{50} values ranging from 5.1 ± 0.2 for compound **2** to 13.9 ± 1.8 μM for compound **7**. All these compounds display predominantly a conformation with the *exo*-methylene group pointing outwards, and H-5 pointing inwards in relation to the twelve-membered ring [8], [11], [12].

Compound **2,** the strongest inhibitor of the NADH oxidase activity, displayed an isobutyrate group at C-7 and a hydroxy group at C-5. The isobutyrate group at C-7 also was a distinct chemical feature of compound **1**, for which the IC_{50} value was 6.3 μM. Less active compounds such as **4** and **7** displayed an acetoxy group at C-7. Even though compound **5** displayed an isobutyrate group at C-7, the presence of an acetoxy group at C-2 and a 2-me-

Table **1** IC_{50} values of jatrophane diterpenes from the latex of *Euphorbia obtusifolia* against NADH oxidase activity[a]

Inhibitor	*NADH oxidase IC_{50} (μM)*
1	6.3 ± 1.4
2	5.1 ± 0.2
3	7.0 ± 3.7
4	13.9 ± 1.6
5	12.7 ± 3.6
6	10.9 ± 2.4
7	13.9 ± 1.8
Rotenone	0.0051 ± 0.0009

[a] Data are means ± SD from four determinations of each compound. The value for rotenone is included for comparison.

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thylbutyrate group at C-5 was paralleled by a decrease of the inhibitory effect on NADH oxidase activity. The structures of the jatrophane derivatives examined here suggest that the presence of acetoxy groups at C-2 and C-7 decrease the inhibitory effect on the NADH oxidase activity. It is thus likely that the antitumor activity of the jatrophane diterpene compounds may be explained by a mechanism associated with inhibition of the mitochondrial electron transport chain, which could result from breakdown of the transmembrane mitochondrial potential ($\Delta\Psi_m$). Breakdown of $\Delta\Psi_m$ is an invariant feature of early apoptosis, and induction of apoptosis is a very promising pathway for which antitumor drugs can be designed.

Material and Methods

The latex of *E. obtusifolia* Poir. var. *obtusifolia* (syn. *E. broussonetii* Willd. ex Link in Buch), was collected in May 1996 from specimens growing in the Valle de Tabares, Tenerife, Canary Islands. The plant material was authenticated by Dr. A. Santos, from the Botanical Acclimatization Garden at La Orotava, Tenerife (voucher number: ORT-33458).

The extraction method was essentially that described by Marco [8]. Briefly, the latex was suspended in boiling MeOH and then cooled to room temperature. This gave rise to a whitish precipitate consisting mainly of waxes and triterpenes, which were eliminated by filtration. The material obtained was subjected to reversed phase column chromatography (CC) and eluted under a slight argon pressure with water, MeOH-water and MeOH. The middle fraction was concentrated under vacuum and extracted with EtOAc. This yielded an oil which was then subjected to CC on silica gel. The fractions were further purified where necessary, by preparative TLC and/or HPLC. This allowed the isolation of the compounds 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 8,9-diacetate 7-isobutyrate 2,5-bis(2-methylbutyrate) $[\alpha]_D^{25}$: +26° ($CHCl_3$, *c* 0.86) (**1**)}; 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 8,9-diacetate 7-isobutyrate 2,3-bis(2-methylbutyrate) $[\alpha]_D^{25}$: +8° ($CHCl_3$, *c* 2.2) (**2**)}; 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 7,9-diacetate 8-benzoate 2,3-bis(2-methylbutyrate) $[\alpha]_D^{25}$: +18° ($CHCl_3$, *c* 0.88) (**3**); 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 7,8,9-triacetate 2-isobutyrate 5-(2-methylbutyrate) $[\alpha]_D^{25}$: +29° ($CHCl_3$, *c* 0.68) (**4**); 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 2,8,9-triacetate 7-isobutyrate 5-(2-methylbutyrate) $[\alpha]_D^{25}$: +32° ($CHCl_3$, *c* 0.68) (**5**); 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 7,8,9-triacetate 2,5-bis(2-methylbutyrate) $[\alpha]_D^{25}$: +23° ($CHCl_3$, *c* 0.78) (**6**); 2,3,5,7,8,9,15-heptahydroxyjatropha-6(17),11-diene-14-one 7,8,9-triacetate 2-nicotinate 5-(2-methylbutyrate) $[\alpha]_D^{25}$: −6° ($CHCl_3$, *c* 0.68) (**7**). All obtained compounds showed at least a 95% purity. Their structures were established by ^{1}H-NMR and ^{13}C-NMR spectral data, aided by NOE measurements and heteronuclear 2D correlations (HMQC, HMBC), as described previously [8].

The bioassays for NADH oxidase activity were essentially those described by Degli et al. [9]; stock solutions (15 mM in absolute ethanol) of the compounds were prepared and kept in the dark at −20 °C. Appropriate dilutions were made before the experiments (maximum ethanol concentrations never exceeded 2% and control activity was not affected by this concentration). After each addition of inhibitor, NADH oxidase activity was measured. Briefly, enzymatic activities were assayed at 22 °C in 50 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA with the SMP from bovine heart diluted to 6 μg/ml. Aerobic NADH oxidation was measured in the presence of 75 μM NADH by following the decrease in absorbance at 340 nm. Rotenone was used as positive control [10].

Data from four titrations under the same conditions were pooled and fitted for graphics. The 50% inhibitory concentration (IC_{50}) was taken as the final compound concentration that yielded 50% inhibition of activity. Data from individual titrations were used to assess the means and standard deviations.

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